DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for *in vitro* Mammalian Chromosomal Aberration Test
(OPPTS 870.5375)

MRID 47185134

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature: Sylvia Milanez
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 21 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for in vitro Mammalian Chromosomal Aberration Test (OPPTS 870.5375)
MRID NO:	47185134
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-TOX-44
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. *In Vitro* Mammalian Chromosomal Aberration Test.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	The information submitted is sufficient to support the requested waiver an *in vitro* mammalian chromosomal aberration test.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for *in vitro* Mammalian Chromosomal Aberration Test (OPPTS 870.5375).

Registrant's Justification

In a reverse mutation study (MRID 47208904), Homobrassinolide Technical at dose levels of 0.03 to 0.05 µg/plate was non-mutagenic in *Salmonella typhimurium* strains TA1537, TA1535, TA98, and TA100.

In a chromosomal aberration test (MRID 47208905), Homobrassinolide Technical did not have the potential to induce chromosome aberrations in mice treated up to a single oral dose of 2000 mg/kg body weight.

In a micronucleus test (MRID 47185127), Homobrassinolide Technical did not have micronucleus induction potential in mice after two days of oral dosing up to a level of 2000 mg/kg body weight.

Based on the results of these three studies, the registrant concluded that Homobrassinolide Technical has no potential for genotoxicity.

Reviewer's Conclusion

The ORNL reviewer for the reverse mutation study cited above found it to be unacceptable. However, the information submitted is sufficient to support the requested waiver for an *in vitro* mammalian chromosomal aberration test.

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for Immunotoxicity (OPPTS 880.3550)

MRID 47185135

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
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Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
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Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L.A. Wilson
Date: FEB 21 2008

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DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for Immunotoxicity (OPPTS 880.3550)
MRID NO:	47185135
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-TOX-45
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. Immunotoxicity.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	The information submitted is not sufficient to support the requested waiver for immunotoxicity testing.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for Immunotoxicity (OPPTS 880.3550).

Registrant's Justification

Homobrassinolide was not a sensitizer to the skin of guinea pigs (MRID 47185124), making it unlikely to show any signs of immunotoxicity.

In a 90-day oral toxicity study in rats (MRID 47208906), the NOAEL for Homobrassinolide Technical was 1000 mg/kg/day. There were no changes in organ weights (e.g., thymus, spleen) or differential white blood cell counts of the treated animals, which would indicate potential interference with normal immune function.

Based on the skin sensitization and subchronic oral toxicity studies, along with results from the acute studies (Table 1), it is very unlikely that Homobrassinolide Technical would produce any immunotoxicity. Additionally, virtually no human exposure is expected to occur after homobrassinolide is applied to target crops.

Table 1. Acute toxicity of Homobrassinolide Technical

Oral LD_{50} (rat)	MRID 47185118	>5000 mg/kg	Toxicity Category IV
Oral LD_{50} (mouse)	MRID 47208903	>5000 mg/kg	Toxicity Category IV
Dermal LD_{50} (rat)	MRID 47185120	>2000 mg/kg	Toxicity Category IV*
Inhalation LC_{50} (rat)	MRID 47185121	2.26 mg/L	Toxicity Category IV
Eye irritation (rabbit)	MRID 47185122	Mild irritant	Toxicity Category III
Skin irritation (rabbit)	MRID 47185123	Not an irritant	Toxicity Category IV
Skin sensitization (guinea pig)	MRID 47185124	Not a sensitizer**	--

*Registrant's classification. ORNL reviewer notes this should be Toxicity Category III

**ORNL reviewer classified this study as unacceptable, but upgradable upon submission of an acceptable positive control study

Reviewer's Conclusion

The ORNL reviewer for the 90-day oral toxicity study in rats found it to be unacceptable, and notes that the thymus and spleen weights were not included in the study report. Based on the finding that the 90-day oral toxicity study is unacceptable, the information submitted is not sufficient to support the requested waiver for immunotoxicity. If the Agency judges the 90-day oral toxicity study to be acceptable, then sufficient information has been submitted to support the waiver for immunotoxicity.

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for Immune Response (OPPTS 880.3800)

MRID 47185140

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature:
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature: Robert H. Ross
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: L. A. Wilson
Date: FEB 21 2008

Disclaimer

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DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for Immune Response (OPPTS 880.3800)
MRID NO:	47185140
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-TOX-50
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. Immune Response.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	The information submitted is not sufficient to support the requested waiver for immune response.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for Immune Response (OPPTS 880.3800).

Registrant's Justification

Homobrassinolide in end use products will be applied to crops in very low amounts (parts per million levels). Its solubility in distilled water is 3.18%, and its other physical and chemical properties suggest it possesses no toxic potential to the environment. Under field conditions, homobrassinolide has been found to be rapidly metabolized in plant tissue, and any residues are rapidly degraded or metabolized.

In acute studies, Homobrassinolide Technical was virtually non-toxic (Table 1).

Table 1. Acute toxicity of Homobrassinolide Technical			
Oral LD_{50} (rat)	MRID 47185118	>5000 mg/kg	Toxicity Category IV
Oral LD_{50} (mouse)	MRID 47208903	>5000 mg/kg	Toxicity Category IV
Dermal LD_{50} (rat)	MRID 47185120	>2000 mg/kg	Toxicity Category IV*
Inhalation LC_{50} (rat)	MRID 47185121	2.26 mg/L	Toxicity Category IV
Eye irritation (rabbit)	MRID 47185122	Mild irritant	Toxicity Category III
Skin irritation (rabbit)	MRID 47185123	Not an irritant	Toxicity Category IV
Skin sensitization (guinea pig)	MRID 47185124	Not a sensitizer**	--

*Registrant's classification. ORNL reviewer notes this should be Toxicity Category III

**ORNL reviewer classified this study as unacceptable, but upgradable upon submission of an acceptable positive control study

The following genetic assays using Homobrassinolide Technical were negative: *Salmonella typhimurium* bacterial mutation (4 standard strains tested up to 0.5 µg/plate, with and without metabolic activation) (MRID 47208904); mammalian (mice) chromosomal mutation (up to 2000 mg/kg) (MRID 47208905); and mammalian (mice) DNA damage (up to 2000 mg/kg) (MRID 47185127).

Homobrassinolide Technical showed minimal toxicity to aquatic organisms. In freshwater bioassays with *Poecilis reticulata*, and *Brachydanio rerio* the 96-hr LC_{50} for Homobrassinolide Technical was 24.56 mg/L and 14.38 mg/L, respectively (MRID 47185129). The 48-hr LC_{50} in *Daphnia magna* was 8.90 mg/L (MRID 47185130).

In a 90-day oral toxicity study in rats (MRID 47208906), the NOAEL for Homobrassinolide Technical was 1000 mg/kg/day. Applying a safety factor of 10 gives an estimated NOAEL of 100 mg/kg/day for a two-year chronic toxicity in rodents. To extrapolate the chronic toxicity NOAEL value of 100 mg/kg/day in rodents to a lifetime exposure value for humans, the 100 mg/kg/day value is multiplied by 60 kg (avg body weight for humans), which would give 6000 mg/day. Dividing the 6000 mg/day value by a safety factor of 10 gives an estimated NOAEL for homobrassinolide exposure in human adults of 600 mg/day. This would be the maximum tolerated dose for human adults. Dividing the adult maximum tolerated dose by a safety factor of 10 gives a maximum tolerated dose of 60 mg/day for special groups, including children.

Homobrassinolide and other brassinosteriods (more than 50 have been identified) are ubiquitous in plants, and are present at concentrations of 10 to 100 µg/kg in pollen, 1 to 100 µg/kg in immature seeds, and 10 to 100 ng/kg in shoots and leaves of various plant species (Kripach, et al., 1999). The homobrassinolide level in plants is about 200 ppb. The average daily intake of

homobrassinolide via dietary sources is expected to be <10 μg/day. Since the tolerated dose in human adults is estimated to be 600 mg/day, and the average daily intake for homobrassinolide is < 10 μg/day, there is an ample margin of safety.

Humans consume plant sterols via food and also as dietary supplements. Since homobrassinolide and other brassinosteroids are biosynthesized from plant sterols (such as campestanol), the consumption of homobrassinolide is considered to be safe.

The homobrassinolide level in plants is about 200 ppb. Assuming the average daily intake of homobrassinolide for humans is 600 mg/kg/day (600 ppm/day), the safety margin can be calculated by dividing 600 ppm by 200 ppb, with the result being 3000-fold safety margin.

Homobrassinolide is a pure chemical, and pure plant chemicals rarely cause hypersensitivity in humans, unlike a plant extract or manufactured chemical.

Reviewer's Conclusion

Much of the justification presented by the registrant is irrelevant. A better justification would be that submitted for the waiver of immunotoxicity (MRID 47185135). However, both the immunotoxicity and immune response requests are supported by the 90-day oral toxicity study in rats. The ORNL reviewer for that study found it to be unacceptable (the bacterial mutation assay cited above was also found unacceptable). Based on that finding, the information submitted is not sufficient to support the requested waiver for immune response. If the Agency judges the 90-day oral toxicity study to be acceptable, then sufficient information has been submitted to support the waiver for immune response.

References Cited

Kripach et al. 1999. In: Brassinosteroids: A New Class of Plant Hormones. Academic Press, New York, NY.

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for Chronic Exposure (OPPTS 870.4100)

MRID 47185141

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
Eric B. Lewis, M.S.

Signature:
Date: FEB 21 2008

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature:
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature:
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Quality Assurance:
Lee Ann Wilson, M.A.

Signature:
Date: FEB 21 2008

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DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for Chronic Exposure (OPPTS 870.4100)
MRID NO:	47185141
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-TOX-51
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. Chronic Exposure.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	The information submitted is not sufficient to support the requested waiver for chronic exposure.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for Chronic Exposure (OPPTS 870.4100).

Registrant's Justification

In acute studies, Homobrassinolide Technical was virtually non-toxic (Table 1).

Table 1. Acute toxicity of Homobrassinolide Technical			
Oral LD_{50} (rat)	MRID 47185118	>5000 mg/kg	Toxicity Category IV
Oral LD_{50} (mouse)	MRID 47208903	>5000 mg/kg	Toxicity Category IV
Dermal LD_{50} (rat)	MRID 47185120	>2000 mg/kg	Toxicity Category IV*
Inhalation LC_{50} (rat)	MRID 47185121	2.26 mg/L	Toxicity Category IV
Eye irritation (rabbit)	MRID 47185122	Mild irritant	Toxicity Category III
Skin irritation (rabbit)	MRID 47185123	Not an irritant	Toxicity Category IV
Skin sensitization (guinea pig)	MRID 47185124	Not a sensitizer**	--

*Registrant's classification. ORNL reviewer notes this should be Toxicity Category III
**ORNL reviewer classified this study as unacceptable, but upgradable upon submission of an acceptable positive control study

The following genetic assays using Homobrassinolide Technical were negative: *Salmonella typhimurium* bacterial mutation (4 standard strains tested up to 0.5 µg/plate, with and without metabolic activation) (MRID 47208904); mammalian (mice) chromosomal mutation (up to 2000 mg/kg) (MRID 47208905); and mammalian (mice) DNA damage (up to 2000 mg/kg) (MRID 47185127).

In a 90-day oral toxicity study in rats (MRID 47208906), the NOAEL for Homobrassinolide Technical was 1000 mg/kg/day. Applying a safety factor of 10 gives an estimated NOAEL of 100 mg/kg/day for a two-year chronic toxicity in rodents. To extrapolate the chronic toxicity NOAEL value of 100 mg/kg/day in rodents to a lifetime exposure value for humans, the 100 mg/kg/day value is multiplied by 60 kg (avg body weight for humans), which would give 6000 mg/day. Dividing the 6000 mg/day value by a safety factor of 10 gives an estimated NOAEL for homobrassinolide exposure in human adults of 600 mg/day. This would be the maximum tolerated dose for human adults. Dividing the adult maximum tolerated dose by a safety factor of 10 gives a maximum tolerated dose of 60 mg/day for special groups, including children.

The 90-day oral study in rats showed no toxicity, no changes in blood chemistry, and no gross or histopathologic lesions. The acute oral LD_{50} in rats was >2000 mg/kg. Since the oral route is the most likely route for human exposure, a chronic human exposure of 2000 mg/kg is highly unlikely.

Homobrassinolide and other brassinosteriods (more than 50 have been identified) are ubiquitous in plants, and are present at concentrations of 10 to 100 µg/kg in pollen, 1 to 100 µg/kg in immature seeds, and 10 to 100 ng/kg in shoots and leaves of various plant species (Kripach, et al., 1999). The average daily intake of homobrassinolide via dietary sources is expected to be <10 µg/day. Since the tolerated dose in human adults is estimated to be 600 mg/day, and the average daily intake for homobrassinolide is < 10 µg/day, there is an ample margin of safety.

Humans consume plant sterols via food and also as dietary supplements. Since homobrassinolide and other brassinosteroids are biosynthesized from plant sterols (such as campestanol), the consumption of homobrassinolide is considered to be safe.

The homobrassinolide level in plants is about 200 ppb. Assuming the average daily intake of homobrassinolide for humans is 600 mg/kg/day (600 ppm/day), the safety margin can be calculated by dividing 600 ppm by 200 ppb, with the result being 3000-fold safety margin.

Reviewer's Conclusion

The registrant did not provide any proof for the statement that homobrassinolide is rapidly metabolized and degraded in plant tissue. Perhaps the strongest support submitted for this waiver is the 90-day oral toxicity study in rats. However, the ORNL reviewer for that study found it to be unacceptable. Based on that conclusion, the information submitted is not sufficient to support the requested waiver for chronic exposure. If the Agency judges the 90-day oral toxicity study to be acceptable, then sufficient information has been submitted to support the waiver for chronic exposure.

Kripach et al. 1999. In: Brassinosteroids: A New Class of Plant Hormones. Academic Press, New York, NY.

DATA EVALUATION RECORD

HOMOBRASSINOLIDE
(Homobrassinolide Technical)

STUDY TYPE: Waiver Request for Carcinogenicity (OPPTS 870.4200)

MRID 47185142

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 07-080

Primary Reviewer:
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Signature: Eric B. Lewis
Date: FEB 21 2008

Secondary Reviewers:
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Signature:
Date: FEB 21 2008

Robert H. Ross, M.S., Group Leader

Signature:
Date: FEB 21 2008

Quality Assurance:
Lee Ann Wilson, M.A.

Signature:
Date: FEB 21 2008

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DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Waiver Request for Carcinogenicity (OPPTS 870.4200)
MRID NO:	47185142
DP BARCODE:	DP347313
DECISION NO:	381556
SUBMISSION NO:	Not provided
TEST MATERIAL:	Homobrassinolide Technical (a.i., 80.0% homobrassinolide)
STUDY NO:	REPAR-HBR-TOX-52
SPONSOR:	Mandava Associates, LLC, 1730 M Street, NW, Suite 906, Washington, DC 20036
TESTING FACILITY:	N/A
TITLE OF REPORT:	Homobrassinolide Technical Biochemical Pesticides Toxicology Data. Carcinogenicity.
AUTHOR:	Mandava, N.B.
STUDY COMPLETED:	June 28, 2007
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed and dated GLP statement was included. The study is not in compliance with the requirements of 40 CFR Part 160.
CONCLUSION:	A carcinogenicity test is not required, and a waiver is therefore not needed.

Product Description

Homobrassinolide Technical is a manufacturing use product intended only for formulation into plant growth regulator end-use products. The active ingredient is 80.0% homobrassinolide. There are no intentionally-added inert ingredients in the product.

Waiver Request

The registrant is requesting a waiver of the data requirement for Carcinogenicity (OPPTS 870.4200).

Registrant's Justification

Homobrassinolide in end use products will be applied to crops in very low amounts (parts per million levels). Its solubility in distilled water is 3.18%, and its other physical and chemical properties suggest it possesses no toxic potential to the environment. Under field conditions, homobrassinolide has been found to be rapidly metabolized in plant tissue, and any residues are rapidly degraded or metabolized.

In acute studies, Homobrassinolide Technical was virtually non-toxic (Table 1).

Table 1. Acute toxicity of Homobrassinolide Technical

Oral LD_{50} (rat)	MRID 47185118	>5000 mg/kg	Toxicity Category IV
Oral LD_{50} (mouse)	MRID 47208903	>5000 mg/kg	Toxicity Category IV
Dermal LD_{50} (rat)	MRID 47185120	>2000 mg/kg	Toxicity Category IV*
Inhalation LC_{50} (rat)	MRID 47185121	2.26 mg/L	Toxicity Category IV
Eye irritation (rabbit)	MRID 47185122	Mild irritant	Toxicity Category III
Skin irritation (rabbit)	MRID 47185123	Not an irritant	Toxicity Category IV
Skin sensitization (guinea pig)	MRID 47185124	Not a sensitizer**	--

*Registrant's classification. ORNL reviewer notes this should be Toxicity Category III

**ORNL reviewer classified this study as unacceptable, but upgradable upon submission of an acceptable positive control study

The following genetic assays using Homobrassinolide Technical were negative: *Salmonella typhimurium* bacterial mutation (4 standard strains tested up to 0.5 µg/plate, with and without metabolic activation) (MRID 47208904); mammalian (mice) chromosomal mutation (up to 2000 mg/kg) (MRID 47208905); and mammalian (mice) DNA damage (up to 2000 mg/kg) (MRID 47185127).

In a 90-day oral toxicity study in rats (MRID 47208906), the NOAEL for Homobrassinolide Technical was 1000 mg/kg/day. Applying a safety factor of 10 gives an estimated NOAEL of 100 mg/kg/day for a two-year chronic toxicity in rodents. To extrapolate the chronic toxicity NOAEL value of 100 mg/kg/day in rodents to a lifetime exposure value for humans, the 100 mg/kg/day value is multiplied by 60 kg (avg body weight for humans), which would give 6000 mg/kg/day. Dividing the 6000 mg/kg/day value by a safety factor of 10 gives an estimated NOAEL for homobrassinolide exposure in human adults of 600 mg/kg/day. This would be the maximum tolerated dose for human adults. Dividing the adult maximum tolerated dose by a safety factor of 10 gives a maximum tolerated dose of 60 mg/day for special groups, including children.

The 90-day oral study in rats showed no toxicity, no changes in blood chemistry, and no gross or histopathologic lesions. The acute oral LD_{50} in rats was >2000 mg/kg. Since the oral route is the most likely route for human exposure, and a chronic human exposure of 2000 mg/kg is highly unlikely, the development of carcinogenicity in humans is also highly unlikely.

Homobrassinolide and other brassinosteriods (more than 50 have been identified) are ubiquitous in plants, and are present at concentrations of 10 to 100 µg/kg in pollen, 1 to 100 µg/kg in immature seeds, and 10 to 100 ng/kg in shoots and leaves of various plant species (Kripach, et al., 1999). The average daily intake of homobrassinolide via dietary sources is expected to be <10

µg/day. Since the tolerated dose in human adults is estimated to be 600 mg/day, and the average daily intake for homobrassinolide is < 10 µg/day, there is an ample margin of safety.

Humans consume plant sterols via food and also as dietary supplements. Since homobrassinolide and other brassinosteroids are biosynthesized from plant sterols (such as campestanol), the consumption of homobrassinolide is considered to be safe.

The homobrassinolide level in plants is about 200 ppb. Assuming the average daily intake of homobrassinolide for humans is 600 mg/kg/day (600 ppm/day), the safety margin can be calculated by dividing 600 ppm by 200 ppb, with the result being 3000-fold safety margin.

Reviewer's Conclusion

The registrant did not provide any proof for the statement that homobrassinolide is rapidly metabolized and degraded in plant tissue. The ORNL reviewers for the 90-day oral toxicity and reverse mutation studies cited above found them to be unacceptable. Regardless, ORNL does not find that carcinogenicity testing is required, and a waiver request is therefore not needed.

References Cited

Kripach et al. 1999. In: Brassinosteroids: A New Class of Plant Hormones. Academic Press, New York, NY.

DATA EVALUATION RECORD

BRASSINOSTEROIDS TECHNICAL

STUDY TYPE: *IN VIVO* MAMMALIAN CYTOGENETICS – ERYTHROCYTE MICRONUCLEUS ASSAY; OPPTS 870.5395 [§84-2]
MRID 47185127

Prepared for

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U.S. Environmental Protection Agency
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Work Assignment #07-080

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Secondary Reviewer: **Date:**_____

DATA EVALUATION RECORD

STUDY TYPE: *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus assay in mouse OPPTS 870.5395 [§84-2]; OECD 474.

EPA Reg. No.: 69361-RT **Product Name**: Brassinosteroids Technical
DECISION: 381556 **DP BARCODE**: 347313

TEST MATERIAL (PURITY): Brassinosteroids Technical (85.1%)

SYNONYMS: None

CITATION: Prabakaran, P. (1998) Homobrassinolide Technical. Biochemical Pesticides Toxicology Data. Micronucleus test in mice. Jai Research Foundation, Valvada 396 108, Dist. Valsad, Gujarat, India. JRF Report No. 273, September, 1998. MRID 47185127. Unpublished.

SPONSOR: Godrej Agrovet Ltd., Pirojshah Nagar, Eastern Express Highway, Vikhroli, Mumbai – 400 079 INDIA.

EXECUTIVE SUMMARY:

In a mouse bone marrow micronucleus assay (MRID 47185127), 6 Swiss albino male mice/dose level were administered (by gavage) brassinosteroids technical (purity 85.1%, batch no. 970004). The test material was given in two daily doses, 24 hours apart, of 0, 500, 1000, or 2000 mg/kg bw, using carboxy methyl cellulose as the vehicle. All animals were sacrificed 24 hours after final treatment. No significant changes in body weights of the treated animals were observed during the period of treatment at any dose, nor were there any clinical signs of toxicity.

The percent of micronucleated erythrocytes in mice treated with the test article up to a limit dose of 2000 mg/kg body weight daily, for two days, did not show any significant increase from the negative vehicle control. The ratio (P/N) of polychromatic erythrocytes (immature cells) to normochromatic erythrocytes (mature cells) did not vary from that of the vehicle control up to the dose level of 1000 mg/kg body weight. The mice treated with the test article at a dose of 2000 mg/kg body weight showed a slight, statistically significant increase ($p<0.05$) in the P/N ratio. The positive control induced the appropriate response. **There was not a significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow after any treatment dose.** It was concluded that the test chemical was negative in this *in vivo* study.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for Test Guideline OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

COMPLIANCE: Signed and dated GLP and Data Confidentiality statements were provided. A

signed and dated Management Statement, equivalent to a Quality Assurance statement was included.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Brassinosteroid Technical

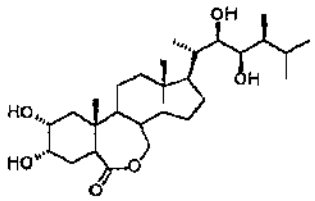
Description: Light yellowish-brown colored powder.

Lot/Batch #: 970004

Purity: 85.1%

CAS # of TGAI: Not reported

Structure:



Brassinosteroids are a group of similar steroidal plant hormones. Brassinolide was the first of these steroid compounds discovered and its structure is shown here.

Solvent Used: Carboxymethyl cellulose

2. Control materials:

Negative control (if not vehicle) :		Final volume:	Route:
Vehicle:	Carboxymethyl cellulose	Final volume: 10 mL/kg bw	Route: oral by metal cannula
Positive control :	Mitomycin-C (MMC), dissolved in distilled water.	Final dose(s): 4 mg/kg bw	Route: i.p., single injection

3. Test animals:

Species:	Mouse			
Strain:	Swiss albino			
Age/weight at study initiation:	Approximately 7 weeks of age / males only: 30 to 35 g			
Source:	Jai Research Foundation			
No. animals used per dose per harvest time	6	Males	0	Females
Properly Maintained?	x	Yes		No

4. Test compound administration:

	Dose levels (mg/kg bw)	Final volume	Route
Rangefinder	No range-finding study was reported.	-	
Main study	0, 500, 1000, 2000	10 mL/kg bw	Oral by metal cannula

B. TEST PERFORMANCE:

1. Treatment and sampling times:

a. Test compound:

Dosing:	[ ]	once	[x]	twice (24 hrs apart)	[ ]	Other			
Sampling (after dose):	[ ]	6 hr	[ ]	12 hr	[x]	24 hr	[ ]	48 hr	[ ] 72 hr
Other:									

b. Negative and/or vehicle control: Carboxymethyl cellulose; same schedule as for test chemical

c. Positive control:

Dosing:	[x]	once	[ ]	twice (24 hrs apart)	[ ]	Other			
Sampling (after dose):	[ ]	6 hr	[ ]	12 hr	[x]	24 hr	[ ]	48 hr	[ ] 72 hr
Other:									

2. Tissues and cells examined:

Were erythrocytes from bone marrow examined?	Yes
No. of polychromatic erythrocytes (PCEs) examined per animal:	≥1000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal:	Range: 629 to 1545
Other	

3. **Details of slide preparation:** The day following the last treatment, mice from all groups were sacrificed by cervical dislocation. Femurs from the animals were recovered and the epicondyle tips removed. The bone marrow cells were expelled by flushing and aspiration into centrifuge tubes using a syringe and 3 mL fetal calf serum. The aspirated bone marrow was mixed using the syringe to dissociate the cells and also to avoid cell clumping. The tubes were centrifuged at 2000 rpm for 10 minutes and the supernatant was discarded, leaving *ca.* 0.5 mL of the serum with the cell pellet. The cell pellet was thoroughly broken up using a pasteur pipette and a drop of the serum with suspended cells was placed on a clean labeled slide. A smear was prepared and allowed to air dry. Two slides were prepared per animal and the cells were fixed with absolute methanol and allowed to air dry. Slides were stained using 5% Giemsa in phosphate buffer (pH 6.8) for 10 minutes, then rinsed in distilled water and air dried.

4. **Evaluation criteria:** Slides were examined for the presence of micronuclei in polychromatic and normochromatic erythrocytes under a microscope. A minimum of 1000 polychromatic erythrocytes (PCEs) were screened per animal and the incidence of micronuclei was recorded, the corresponding number of normochromatic erythrocytes (NCEs) with and without micronuclei was also recorded. Of the two slides prepared per animal, one slide was used for screening micronucleated erythrocytes and the other slide was kept in reserve. The per cent micronucleated erythrocytes and the ratio between PCE and NCE (P/N) were calculated.

5. **Statistical methods:** The data on body weight, % micronucleated erythrocytes and P/N ratio were analyzed statistically, using the Student's t-test.

II. REPORTED RESULTS:

A. PRELIMINARY TOXICITY ASSAY:

No rangefinder study was reported for the micronucleus assay. All that is known is that the highest dose used, which was administered orally by metal cannula to males only, was the limit dose for the assay of 2000 mg/kg bw, and that the mice were weighed daily during the two days of treatment and then just prior to sacrifice on the third day. The mice were also observed for clinical signs of toxicity and mortality daily for three days. No significant changes in body weights of the treated animals were observed during the period of treatment at any dose level. There were no clinical signs of toxicity observed at any dose level or at any time during the study.

B. MICRONUCLEUS ASSAY:

The percent of micronucleated erythrocytes in mice treated with brassinosteroids technical up to a dose level of 2000 mg/kg body weight daily, for two days, did not show any significant increase from that of the control (Table 1). The P/N ratio did not vary from that of control up to the dose level of 1000 mg brassinosteroids technical/kg body weight. The mice treated with 2000 mg brassinosteroids technical/kg body weight showed a slight, statistically significant increase ($p<0.05$) in the P/N ratio. The bone marrow cells from the treated mice did not show any abnormal chromosome figures or large-sized micronuclei. The results of the positive control (4 mg mitomycin C/kg body weight) gave the expected results, indicating that the micronucleus test was working properly.

TABLE 1. Summary of micronucleus assay with brassinosteroids technical in bone marrow of male mice.

	Total erythrocytes		Mean % MNE ± SD	Mean PCE [b] /NCE [c] ratio ± SD
	Scored	MNE [a]		
Vehicle control (carboxymethyl cellulose)	11649	3	0.027 ± 0.029	1.318 ± 0.500
Brassinosteroids Technical 500 mg/kg bw	11335	6	0.053 ± 0.035	1.187 ± 0.235
1000 mg/kg bw	11041	5	0.045 ± 0.039	1.292 ± 0.277
2000 mg/kg bw	9488	6	0.063 ± 0.038	1.887 ± 0.444 *
Positive control (MMC 4 mk/kg b.w.)	13999	91	0.657 ± 0.294 *	0.833 ± 0.116

Data obtained from MRID 47185127, Table 2 on page 19.
[a] MNE = micronucleated erythrocytes (includes mature and immature cells)
[b] PCE = polychromatic (immature) erythrocytes
[c] NCE = normochromatic (mature) erythrocytes
*Statistically significantly higher than vehicle control, $p < 0.05$ by Student's t-test.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATOR'S CONCLUSIONS:

The investigator concluded that under the test conditions used, brassinosteroids technical does not induce micronuclei in bone marrow cells of mice.

B. REVIEWER COMMENTS:

Presentation of the methods used and discussion of the results obtained were minimal in this report. However, based on the data presented, the reviewer agrees with the investigator's conclusions. The test article was studied up to the limit dose required by the Guidelines. All three doses of the test article produced a higher frequency of micronucleated erythrocytes than found in the negative control, but none of these frequencies reached statistical significance, nor was there a dose-related increase in the frequency of micronucleated erythrocytes.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement for OPPTS 870.5395; OECD 474 for *in vivo* cytogenetic mutagenicity data.

C. STUDY DEFICIENCIES:

Female mice were not used in this study. However, no data or references were presented to show that there was no substantial difference in toxicity of the test article between the sexes. No justification was given for the use of carboxymethyl cellulose as the vehicle. The stability of the neat test article and its stability in the vehicle were not reported. The criteria for a positive response were not stated.